IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Cuttitta *et al.*
Application No. 10/571,012
Filed: March 8, 2006
Confirmation No. 4600
For: NON-PEPTIDE ANTAGONISTS OF GASTRIN RELEASING PEPTIDE
Examiner: Anna Pagonakis
Art Unit: 1628
Attorney Reference No. 4239-82094-06

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DECLARATION OF DR. FRANK CUTTITTA UNDER 37 C.F.R. § 1.132

I, Frank Cuttitta, Ph.D., declare as follows:

1. I am an inventor listed on U.S. Patent Application No. 10/571,012, filed March 8, 2006. I have read and am familiar with this application (hereinafter, "the subject application").

2. I hold a Ph.D. in Microbiology/Immunology from the University of Maryland. I am the Director of the NCI Angiogenesis Core Facility in Gaithersburg, Maryland. My *curriculum vitae* was made of record as part of the Declaration submitted with the Request for Continued Examination on September 10, 2010 (hereinafter "the September 2010 Declaration"). By virtue of my education, training, and professional experience, I am knowledgeable about the biology of cancer, angiogenesis, gastrin releasing peptide (GRP), and the identification and activity of agonists and antagonists of GRP function.

3. I have read the Office Action dated May 26, 2011 and the references cited therein.

4. I understand that the Office continues to assert that the claims of the subject application are allegedly anticipated by Japanese Patent No. 10212235 (hereafter JP10212235). Specifically, the Office continues to assert that by describing Compound I as an "anti-tumor

compound," JP10212235 necessarily describes Compound I (and its species) as "inhibiting an activity of GRP."

5. In the Declaration submitted with the Request for Continued Examination on April 28, 2011 (hereinafter "the April 2011 Declaration"), I explained why one of skill in the art would not necessarily equate every compound described as an "anti-tumor compound" with a compound that can inhibit an activity of GRP (*see* ¶¶5.1-5.3, 7.1, 7.3 and 7.4). I further note that none of the compounds tested for anti-tumor activity in JP10212235 is a known GRP inhibitor. Moreover, Compound 77427 (labeled Compound 105 in JP10212235) is not used in any experiment in JP10212235, and has a substantially different chemical structure from any of the compounds that were used in the experiments presented in JP10212235 (*see* Exhibit DD, submitted with the Request for Continued Examination on April 28, 2011).

6. Previously, I described the identification of Compound 77427 as a small molecule mimetic of the GRP functional antagonist, monoclonal antibody 2A11 (*see* ¶8 of the September 2010 Declaration). I also previously noted that "just as changes in an antibody's peptide structure may significantly affect its binding specificity, so too changes in chemical structure of a small molecule mimetic will affect its activity" (*see* ¶7.6 of the April 2011 Declaration). Thus, notwithstanding the assertions of JP10212235 or the Office, one of skill would not infer any property of Compound 105 from any of the other species of Compound I. Quite the contrary, in the absence of data to demonstrate a specific shared biological property, one of skill in the art would assume that compounds of different structure will possess different biological activities.

7. To test this assumption for Compound 77427, two additional compounds were recently assayed in my lab for specific binding to GRP (as determined by the ability to block the interaction between monoclonal antibody 2A11 and GRP). The first compound tested, NSC 619198 is similar to NSC 77427 (Compound 77427), and is identical to Compound 109 of JP10212235. The second compound tested, NSC 636346, is not described in JP10212235, but was randomly selected from the National Cancer Institute Developmental Therapeutics Program (DTP) pool of small molecules containing ring structures. Additionally, Compound 77427 and bovine serum albumin (BSA) were used as positive and negative controls, respectively.

8. As noted above, the affinity of each compound for GRP was tested by assessing the ability of the compound of interest to disrupt the binding of the GRP neutralizing antibody 2A11 to solid-phased GRP. The results of this assay are attached as Exhibit EE. The procedure followed for this assay was substantially the same as the primary screen for GRP modulating compounds described in the subject specification in Example 3 (*see* Specification, at page 33, lines 9-27) and is summarized briefly as follows:

9. Solid phased GRP was produced by adding 100ng GRP/50µl, 50ng GRP/50 µl or 25ng GRP/50 µl in each well of a 96-well polyvinyl chloride (PVC) plate. The plates were incubated at room temperature for 30 minutes on a rotary shaker. After the incubation, 140µl of 1% BSA in PBS was added to each well and the plates were further incubated at room temperature for 30 minutes on a rotary shaker. The solution was then aspirated from each well, which were washed twice with PBS.

10. Compounds were obtained from the National Cancer Institute DTP, and tested for the ability to block the binding of the GRP neutralizing antibody 2A11 to the solid phased GRP. Specification sheets for each of the compounds tested are attached as Exhibit FF. Each test compound was evaluated at 10 µM concentration and either incubated with the solid phased GRP separately from MoAb 2A11 (at 1:1000 dilution of 1 mg/ml) or incubated jointly with the antibody.

11. When incubated separately, 50 µl of the test compound (10 µM in 0.1% DMSO) were first added to the wells of the plate, and incubated for 30 minutes at room temperature on a rotary shaker, followed by two 50 µl washes with PBS. 50 µl of MoAb2A11 (1:1000 dilution) were then added to each well and incubated for 30 minutes at room temperature on a rotary shaker. When incubated jointly, 25 µl of the test compound (20 µM in 0.1% DMSO) were added to each well along with 25 µl MoAb2A11 at a 1:500 dilution. Thus, the final concentrations of test compound and antibody in each well were 10 µM test compound and 1:1000 dilution MoAb 2A11. The plates were then incubated for 30 minutes at room temperature on a rotary shaker, followed by two 50 µl washes with PBS.

12. To detect antibody binding to GRP, 50 µl of a 1:500 dilution of Goat Anti-IgG Mouse – HRP (horseradish peroxidase) was added to each well and incubated for 30 minutes at room temperature on a rotary shaker. The solution in each well was aspirated, and each well was washed twice with PBS. 100 µl of chromogen (Invitrogen's ready-to-use stabilized 3,3',5,5'-tetramethybenzidine (TMB) solution) was added and incubated for 30 minutes in the dark at room temperature. 50 µl acid solution (1N HCl) was added to develop the chromogen color. Soluble product in each well was determined by reading absorbance at 450 nm.

13. Exhibit EE presents three charts, each of which shows the average of twelve assays of each indicated compound (last three numbers are shown), using solid phased GRP at 100 ng/50 µl, 50 ng /50 µl or 25 ng/50 µl. Each chart also indicates whether the test compound was added separate from or jointly with the antibody. At all three GRP concentrations, NSC 77427 significantly inhibits antibody binding ($p<0.001$), as shown by the reduction in light absorbance in comparison to the BSA negative control. In contrast, neither of the other test compounds significantly inhibited antibody binding in comparison to BSA.

14. The results presented in Exhibit EE demonstrate that the moderate difference in structure between NSC 77427 and NSC 619198 produces a **significant** change (reduction) in the ability of NSC 619198 to bind to GRP. I would not expect other species of Compound I with greater differences in structure from Compound 77427 to "regain" any ability to bind to GRP. This observation also demonstrates that notwithstanding any of the assertions or data presented in JP10212235, the ability to bind to GRP and affect a GRP activity is not a universal, necessary characteristic of all species of Compound I.

15. I hereby declare that all statements made herein are of my own knowledge, are true and that all statements made on information and belief are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

10/25/11
Date

Name Frank Cuttitta, Ph.D.